

Stavrianopoulos et al.

Serial No.: Not Yet Known

(Divisional of S.N. 10/096,075, filed March 12, 2002)

Filed: Herewith

Page 3 [Preliminary Amendment (Accompanying Divisional Application
Under 37 C.F.R. §1.53(b)) --- January 23, 2004]

PLEASE AMEND THIS APPLICATION AS FOLLOWS:

In The Title:

Change the title of the invention to:

-- PROCESS FOR DETECTING THE PRESENCE OR QUANTITY OF
ENZYMATIC ACTIVITY IN A SAMPLE --

In The Claims:

Please cancel claim 1.

Please add new claims 287-321 as follows:

Claim 1 (Canceled Herein)

Claims 2-286 (Previously Canceled)

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287. (NEW) A dye composition of the formula

R – Fluorescent Dye

wherein R is covalently linked to said Fluorescent Dye comprises two or more members in combination from

- a) unsaturated aliphatic groups;
- b) unsaturated heterocyclic groups;
- c) aromatic groups;

and wherein R is capable of providing a conjugated system or an electron delocalized system with said fluorescent dye.

288. (NEW) The dye composition of claim 287, wherein said unsaturated aliphatic groups comprise an alkene or an alkyne.

289. (NEW) The dye composition of claim 287, wherein said aromatic groups comprise a phenyl group, an aryl group or an aromatic heterocyclic group.

290. (NEW) The dye composition of claims 288 or 289, wherein said unsaturated aliphatic groups or aromatic groups are substituted.

291. (NEW) The dye composition of claim 290, wherein said substituted unsaturated aliphatic groups or substituted aromatic groups comprise alkyl groups, aryl groups, alkoxy groups, phenoxy groups, amines, amino groups, amido groups, carboxy groups, nitrates, nitrites, sulfonates, sulfhydryl groups or phosphates.

292. (NEW) The dye composition of claim 290, wherein said substituted aromatic groups comprise a fused ring structure.

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293. (NEW) The dye composition of claim 292, wherein said fused ring structure is a naphthalene, anthracene or a phenanthrene.

294. (NEW) The dye composition of claim 287, wherein said combination comprises two members of the same group or of different groups.

295. (NEW) The dye composition of claim 294, wherein said different groups comprise

an unsaturated aliphatic group (a) and an unsaturated heterocyclic group (b);

an unsaturated aliphatic group (a) and an aromatic group (c); or

an unsaturated heterocyclic group (b) and an aromatic group (c).

296. (NEW) The dye composition of claim 287, wherein said fluorescent dye comprises an anthracene, a xanthene, a cyanine, a porphyrin, a coumarin or a composite dye.

297. (NEW) The dye composition of claim 287, further comprising a charged or polar R' group.

298. (NEW) The dye composition of claim 297, wherein said charged or polar R' group increases aqueous solubility of said composition.

299. (NEW) The dye composition of claim 288 or 297, further comprising a reactive group R_x attached to either said fluorescent dye, said R group or said R' group.

Enz-61(D12)

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300. (NEW) The dye composition of claim 299, further comprising a linker arm attaching said reactive group R_x to said fluorescent dye, said R group or said R' group.

301. (NEW) The dye composition of claim 299, wherein said reactive group R_x comprises sulfhydryl, hydroxyl, amine, isothiocyanate, isocyanate, monochlorotriazine, dichlorotriazine, mono- or di-halogen substituted pyridine, mono- or di-halogen substituted diazine, maleimide, aziridine, sulfonylhalide, acid halide, hydroxysuccinimide ester, hydroxysulfosuccinimide ester, imidoester, hydrazine, azidonitrophenyl, azide, 3-(2-pyridyl dithio)-propionamide, glyoxal or aldehyde.

302. (NEW) The dye composition of claim 300, wherein said reactive group R_x comprises sulfhydryl, hydroxyl, amine, isothiocyanate, isocyanate, monochlorotriazine, dichlorotriazine, mono- or di-halogen substituted pyridine, mono- or di-halogen substituted diazine, maleimide, aziridine, sulfonylhalide, acid halide, hydroxysuccinimide ester, hydroxysulfosuccinimide ester, imidoester, hydrazine, azidonitrophenyl, azide, 3-(2-pyridyl dithio)-propionamide, glyoxal or aldehyde.

303. (NEW) The dye composition of claim 299, wherein as a reactive group R is capable of forming a carbon-carbon linkage with a target.

304. (NEW) The dye composition of claim 300, wherein as a reactive group R is capable of forming a carbon-carbon linkage with a target.

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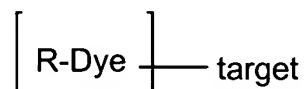
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305. (NEW) The dye composition of claim 303, wherein said reactive group R comprises an alkene group, an alkyne group, a halogenated compound or a metallo-organic compound.

306. (NEW) The dye composition of claim 304, wherein said reactive group R comprises an alkene group, an alkyne group, a halogenated compound or a metallo-organic compound.

307. (NEW) A labeled target having the structure



wherein said Dye is a fluorescent dye, wherein R is covalently linked to said Dye, and wherein R comprises two or more members in combination from

- a) unsaturated aliphatic groups;
- b) unsaturated heterocyclic groups;
- c) aromatic groups;

and wherein R is capable of providing a conjugated system or an electron delocalized system with said Dye.

comprises two or more unsaturated aliphatic groups, unsaturated heterocyclic groups, aromatic groups, or combinations of the foregoing groups and wherein R is covalently attached to said fluorescent dye and is capable of providing a

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conjugated system or an electron delocalized system with said fluorescent dye, and wherein said target is covalently attached to said Dye or said R.

308. (NEW) The labeled target of claim 307, wherein said unsaturated aliphatic groups comprise an alkene or an alkyne.

309. (NEW) The labeled target of claim 307, wherein said aromatic groups comprise a phenyl group, an aryl group or an aromatic heterocyclic group.

310. (NEW) The labeled target of claims 308 or 309, wherein said unsaturated aliphatic groups or aromatic groups are substituted.

311. (NEW) The labeled target of claim 307, wherein said substituted unsaturated aliphatic groups or substituted aromatic groups comprise alkyl groups, aryl groups, alkoxy groups, phenoxy groups, amines, amino groups, amido groups, carboxy groups, nitrates, nitrites, sulfonates, sulfhydryl groups or phosphates.

312. (NEW) The labeled target of claim 310, wherein said substituted aromatic groups comprise a fused ring structure.

313. (NEW) The labeled target of claim 312, wherein said fused ring structure is a naphthalene, anthracene or a phenanthrene.

314. (NEW) The labeled target of claim 307, wherein said combination comprises two members of the same group or of different groups.

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315. (NEW) The labeled target of claim 314, wherein said different groups comprise

an unsaturated aliphatic group (a) and an unsaturated heterocyclic group (b);
an unsaturated aliphatic group (a) and an aromatic group (c); or
an unsaturated heterocyclic group (b) and an aromatic group (c).

316. (NEW) The labeled target of claim 307, wherein said fluorescent dye comprises an anthracene, a xanthene, a cyanine, a porphyrin, a coumarin or a composite dye.

317. (NEW) The labeled target of claim 307, further comprising a charged or polar R' group

318. (NEW) The labeled target of claim 307, wherein said charged or polar R' group increases aqueous solubility of said composition.

319. (NEW) The labeled target of claim 317, further comprising a linker arm attaching said target to said fluorescent dye, said R group or said R' group.

320. (NEW) The labeled target of claim 307, wherein said target comprises a protein, a peptide, a nucleic acid, a nucleotide or a nucleotide analog, a receptor, a natural or synthetic drug, a synthetic oligomer, a synthetic polymer, a hormone, a lymphokine, a cytokine, a toxin, a ligand, an antigen, a hapten, an antibody, a carbohydrate, a sugar or an oligo- or polysaccharide.

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321. (NEW) The labeled target of claim 320, wherein said ligand comprises biotin, iminobiotin, digoxigenin or fluorescein, and the dye comprises a fluorescent dye.

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